Primer

The Golgi apparatus

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At some point in their lives all cells need to grow and divide. In eukaryotic cells these processes require the delivery of new proteins and lipids from their site of synthesis in the endoplasmic reticulum (ER) to the cell surface. The Golgi apparatus, first visualised under the light microscope in 1898 by Camillo Golgi using a staining method he had developed to study neurons, lies on the route between the ER and other destinations within the cell. The electron microscopic (EM) studies of Felix and Dalton in the 1950s revealed that the Golgi apparatus is comprised of a series of overlapping cisternal or plate-like, membrane structures and associated 60 to 70 nm diameter vesicles. Palade and co-workers then went on to demonstrate from their studies of pancreatic cells that secreted proteins pass through the Golgi apparatus on their way to the cell surface or other destinations within the cell such as the lysosomes. This layered cisternal structure is often called a stack, and has a defined polarity, *cis*- being the face accepting material from the ER, and *trans*- being the face where material exits for secretion.

The function of the Golgi apparatus

The Golgi apparatus has two key functions, and could be seen as a combined assembly line and logistics centre. It houses the enzymes responsible for the synthesis of complex carbohydrate structures found on many proteins and lipids, while both the *cis*- and *trans*-faces of the Golgi apparatus are important sites for the sorting of proteins and lipids for delivery to specific subcellular destinations.

Complex carbohydrate modifications in eukaryotes are compartmentalised into the ER and Golgi apparatus. Proteins can be glycosylated in two different ways, *N*-linked glycosylation on asparagine residues, and *O*-linked glycosylation on serine or threonine residues. *N*-linked glycosylation is initiated in the ER, with the transfer of a branched sugar structure from the isoprenoid lipid dolichol to specific asparagine residues, while *O*-linked glycosylation is initiated in the Golgi. The exact series of sugar modifications in both cases depends not only on the species, but the tissue, cell type and even growth state of the cell under investigation. Complex carbohydrate processing continues in the Golgi apparatus, where sugar transferases and sugar-trimming enzymes add or remove a variety of sugars at various stereospecific positions. These enzymes are localised to discrete cisternae within the Golgi apparatus, in an order that corresponds to their sequence of action. The Golgi apparatus could therefore be viewed as an assembly line for the production of correctly glycosylated proteins.

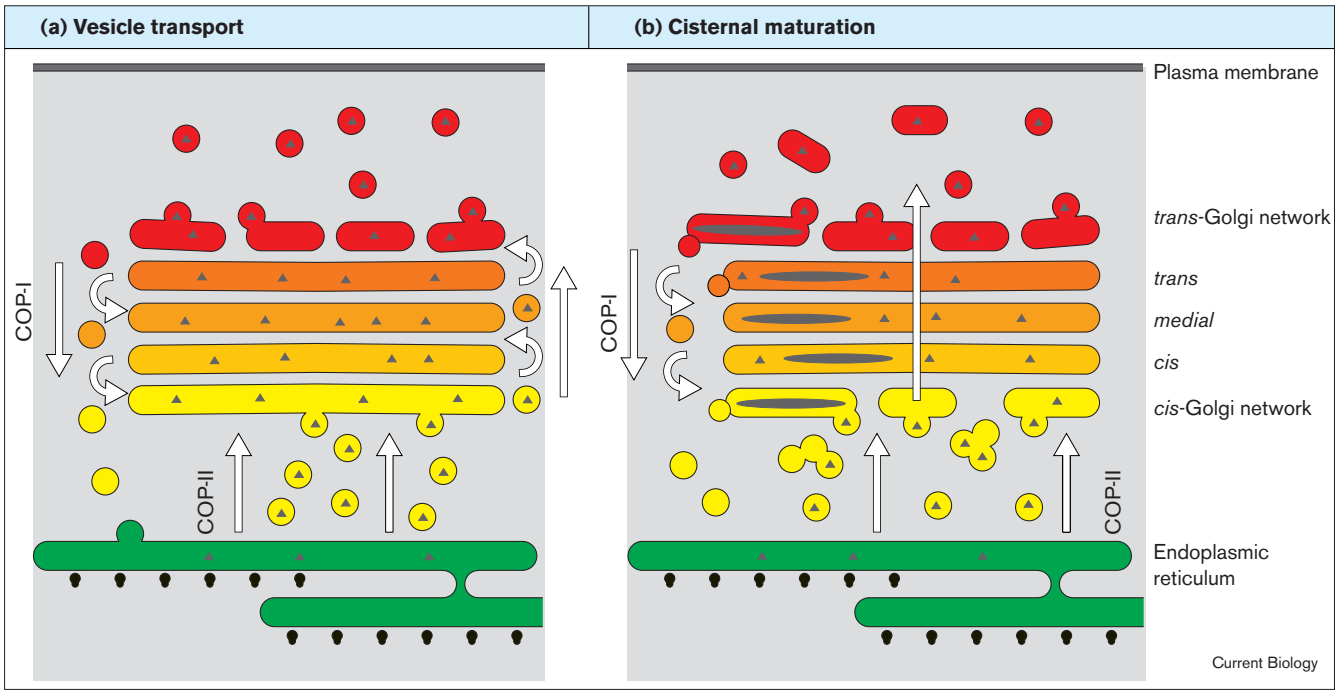
The first, or *cis*-Golgi cisterna is a major site for the sorting of secretory and ER-derived proteins. Many ER proteins contain the carboxy-terminal sequence KDEL, which is recognised by an integral membrane receptor in the Golgi and results in the packaging of these molecules in vesicles for transport back to the ER. Other signals are found in the cytoplasmic domains of transmembrane proteins. The di-lysine motif is one such signal found at the carboxyl terminus of membrane proteins. It binds directly to the proteinaceous vesicle coat termed COP-I, causing proteins that bear di-lysine to become concentrated in vesicles destined for the ER. The exact mechanism by which COP-I recognises and decodes sorting signals of proteins is highly complex, as it appears to discriminate between related signals specifying either forward or retrograde transport through the Golgi apparatus. A series of recent studies indicate that COP-I in conjunction with the small GTPase ARF1 and its specific GTPase-activating protein (ARF-GAP) operate a kinetic proofreading mechanism for signal decoding. How this would result in the formation of two classes of COP-I vesicle going in different directions is unclear.

Finally, at the *trans*-face of the Golgi, is the *trans*-Golgi network (TGN) where proteins are sorted for delivery to their final subcellular destination. Proteins destined for the endocytic pathway, such as lysosomal enzymes, are bound by a receptor in the TGN which recognises a specific sugar modification on these proteins, mannose-6-phosphate, and are segregated into forming vesicles covered with the clathrin coat protein. Other transport events at the TGN are less well understood, the exact nature of the transport intermediates formed and the coat proteins, if any, that cover them remaining mysterious.

Transport mechanisms

Despite many years of intensive research there is no definitive answer to how secretory cargo is transported through the Golgi apparatus, and it is therefore still an area of some controversy. Two mechanisms for cargo transport have been proposed, referred to as the cisternal maturation and vesicle transport models (Figure 1). Since the groundbreaking work of Palade and colleagues in the 1960s, the vesicle transport model has held centre stage, although the cisternal maturation model actually pre-dates it by a decade. The current versions of both models envisage secretory cargo exiting the endoplasmic reticulum in some form of vesicular carrier, but even at this early point the two models then diverge. The vesicle transport model proposes that the Golgi cisternae are stable pre-existing structures through which the cargo molecules pass.

Figure 1

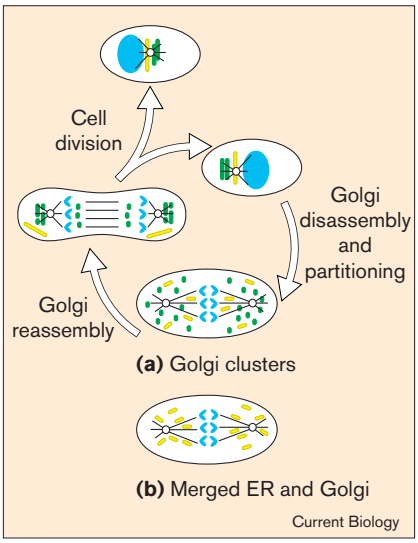


Transport through the Golgi apparatus. **(a)** Vesicle transport envisages stable cisternae between which cargo molecules are transported in small vesicular carriers in a vectorial fashion. **(b)** Cisternal maturation proposes that the first Golgi cisterna is created *de novo* from ER-derived vesicles, and then matures by exchanging enzymes and other components of the Golgi, but not secretory cargo, both with other cisternae and with the ER. The Golgi apparatus cisternae are colour coded to indicate their unique but related composition.

Vesicles coming from the endoplasmic reticulum recognise and fuse with the first, or *cis*-Golgi, cisterna and deliver their content. This content can then undergo one of a number of fates: Golgi enzymes are retained if the cisterna represents their final destination, while secretory cargo molecules get packaged into vesicles destined for the next cisterna in the Golgi stack. Escaped ER proteins and structural components such as the SNAREs and putative cargo receptors involved in ER-to-Golgi traffic are packaged into vesicles destined for the ER. At the *trans*-most face of the Golgi, the *trans*-Golgi network, proteins and lipids are packed into a variety of classes of vesicle destined for different destinations within the cell, such as the endosomal system and the plasma membrane. Much of the evidence supporting the vesicle transport model comes from the early studies of Palade and co-workers who provided both morphological and biochemical evidence for the vectorial transport of secretory proteins through the Golgi apparatus in vesicular carriers. Latterly, the work of Rothman and colleagues has reconstituted this process *in vitro*, and allowed the identification of both membrane and cytosolic proteins required for vesicle formation and fusion. This work culminated in the isolation of Golgi transport vesicles and the characterisation of COP-I, which is comprised of seven polypeptides and the small GTPase ARF1. Subsequently it has been demonstrated that these vesicles appear to function as highly selective bi-directional carriers of material through the Golgi apparatus, and from the Golgi apparatus to the ER. They require the Golgi tethering factor p115, plus the *N*-ethyl maleimide sensitive fusion factor (NSF) and its cofactors in order to recognise and fuse with their target membrane. Small coiled-coil transmembrane proteins, the SNAREs, originally identified as receptors for NSF, mediate the fusion of the lipid bilayers of the vesicle and target membranes.

The cisternal maturation hypothesis proposes that ER-derived vesicles contain all the necessary components to build a new Golgi *cis*-cisterna. This new cisterna then matures by exchanging proteins and lipids by the process of vesicle transport with the ER and other later Golgi cisterna. Cargo molecules are not transported in vesicles in this model, only molecules such as the enzymes that process the cargo, and structural components of the Golgi. When a cisterna reaches its final maturation state at the *trans* face of the Golgi it is then broken down, and the Golgi

Figure 2



In animal cells the Golgi apparatus breaks down into small vesicles as the cell enters mitosis, after which point two models have been proposed to explain its inheritance. **(a)** These vesicles then partition between the two daughter cells, clustering around the spindle poles in a microtubule-dependent manner. In telophase, these vesicles then fuse to reform a Golgi apparatus. **(b)** Alternatively, they may merge with the ER, which is dispersed throughout the cell. As the cell exits mitosis, Golgi stacks reform at sites where vesicles form from the ER. In either case, each daughter cell inherits the components necessary to build a functional Golgi apparatus. The Golgi apparatus is indicated in green, the ER in yellow, and the DNA in blue.

components recycled. Some cargo molecules are packaged into vesicles for delivery to their final destination, while the remnants of the cisternae function as cargo carriers and deliver their contents to the plasma membrane. Cisternal maturation has one clear advantage over the vesicle transport model. It explains how large structures, such as scales in primitive algae and extracellular matrix molecules that are simply too big to fit into 60 to 70 nm diameter vesicles, can move through the Golgi apparatus. Many cell biologists once sceptical of cisternal maturation were converted by the recent study of Luini and his co-workers on the transport of procollagen through the Golgi apparatus, demonstrating its presence in maturing cisternal elements rather than vesicles. In this model, the COP-I vesicle is proposed to function as a retrograde carrier, recycling enzymes and other Golgi components either back to the ER or to a previous Golgi cisterna.

A final twist that can be applied to both models is that transient tubular connections, rather than vesicles, might mediate transport between the different layers of the Golgi stack. This tubule-based transport mechanism has also been proposed to operate between the Golgi apparatus and the ER.

Cell-cycle regulation

As long ago as 1924 it had been observed during mitosis that the Golgi apparatus in animal cells loses its characteristic ribbon-like morphology and breaks down into many clusters of vesicles. These vesicles then partition between the two daughter cells and reassemble to produce a functional Golgi apparatus in each cell. These changes are accompanied by a block in secretion during mitosis, which has now been linked to the molecular mechanism of Golgi disassembly. It is thought that the mitotic kinase, Cdc2–cyclin B, phosphorylates components of the Golgi vesicle docking machinery such that vesicles can no longer find and recognise their target membrane. Vesicle formation is unaffected, with the net result that the Golgi apparatus is reduced to vesicular structures. It is thought that other events are also required to permit this vesiculation, such as the dismantling of structural complexes holding adjacent cisternae together which are also regulated by mitotically active kinases.

The exact mechanism of Golgi apparatus partitioning to the two daughter cells is currently hotly disputed (Figure 2). One camp holds that the Golgi remains as discrete vesicles during this process, which partition in a microtubule-dependent manner to the spindle poles of the dividing cell. The other claims that Golgi membranes are recycled into the ER during the mitotic disassembly process, and that it is the ER which is dispersed between the two daughter cells along microtubules. The Golgi apparatus is then proposed to rebuild at sites where secretory vesicles form from the ER, the so-called ER exit sites. There is compelling evidence for both views, suggesting that aspects of both need to be taken into account. It is quite possible that some Golgi proteins are recycled back into the ER during mitosis, while others are not and remain in some kind of template structures necessary to rebuild a functional Golgi apparatus.

All the apparent controversies relating to the Golgi apparatus perhaps show that this fascinating organelle is still very poorly understood. Recent discoveries such as the localisation to the Golgi apparatus of molecules triggering apoptotic cell death simply highlight this. Many questions still remain completely unanswered or even uninvestigated, perhaps the most fascinating being how did the Golgi apparatus evolve to its present complexity.

Key references

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